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14. ABSTRACT

Tissue Factor (TF) is the cell surface receptor that activates coagulation by binding the serine protease coagulation factor VIIa (VIIa). The activation of the coagulation cascade leads to thrombin generation, fibrin formation and platelet activation which together aide tumor growth and metastasis.While the role of TF in metastasis through thrombin pathways is well established, evidence is increasing that TF may drive tumor development dependent on cell signaling pathways. A newly developed breast cancer model with a tetracycline regulated TF expression cassette shows TF enhances breast cancer tumor growth. This model will be useful to mechanisms by which TF enhances breast cancer progresssion. In this grant, we further evaluated the role of the TF cytoplasmic domain in breast cancer progresssion. We established tumor prone transgenic models in the C57Bl/6 background and compared tumor development in TF cytoplasmic domain deleted mice with wild-type animals. Consistent with a recent report, we found that the C57Bl/6 C3-TAg model is unsuitable for studying breast cancer, because mice developed debilitating chondromatosis prior to the appearance of breast tumors. Experiments are ongoing to evaluate the role of the TF cytoplasmic domain in breast cancer development and progression to metastatic disease in the PyMT model.

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Introduction

Tissue Factor (TF) is the cell surface receptor that activates coagulation by binding the serine protease coagulation factor VIIa (VIIa) . The activation of the coagulation cascade leads to thrombin generation, fibrin formation and platelet activation which together may aide tumor growth and metastasis. How TF signaling pathways influence tumor progression is poorly understood. This grant specifically addressed the question whether the TF cytoplasmic domain acts as a brake of breast cancer progression or whether the TF cytoplasmic domain participates in tumor progression, utilizing TF cytoplasmic domain deleted mice as a rigorous genetic model.

Body

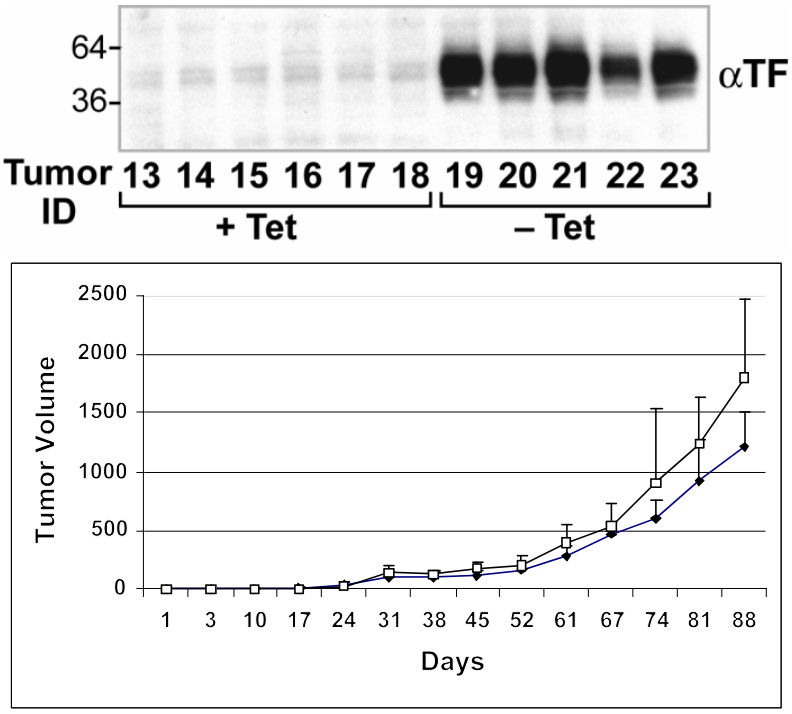

Fig. 1:TF expression leads to enhanced tumor growth of breast cancer cells. Tet-regulated TF-expressing BMS cells were injected subcutaneously into mice. Top panel: tumors from untreated or tetracycline treated mice were lysed and homogenized in detergent and TF was immunoprecipitated from the homogenate. Expression of TF was determined by Western Blot. Lower panel: Tumor growth was followed for 3 months in mice receiving tetracyclin to suppress TF expression (black dots), or in untreated controls (white squares).

This application has two specific aims. Aim 1 is to analyze the role of the TF cytoplasmic domain by transfecting TF negative breast cancer cells. Preliminary data with melanoma cells showed that transfection with full-length, but not cytoplasmic domain deleted TF suppressed tumor growth, indicating a regulatory role of the TF cytoplasmic domain in certain tumor cells. In response to the suggestions of the review committees, we identified TF negative breast cancer cells and transfected these cells with human TF under the control of tetracycline regulated promoters. Tumors cells were injected subcutaneously into immunodeficient Scid/Scid mice. Mice were treated with or without tetracycline in their drinking water. Tetracycline administration

lead to the expected loss of human TF expression in the tumors. Contrary to the melanoma model, TF expression enhanced tumor growth of breast cancer cells ($p < 0.05$ using a two-tailed t-test). We could not detect phosphorylation of the TF cytoplasmic domain in these tumors and conclude that there is no apparent role for the TF cytoplasmic domain in suppressing tumor development in this breast cancer model. However, we have recently found TF phosphorylation in primary isolates of breast cancer and continue a clinical collaboration to evaluate the prognostic predictive value of this posttranslational modification of TF. Remaining tasks on Aim 1 were to test whether overexpression of WW-domains can release suppression of tumor growth by the TF cytoplasmic domain which was not feasible in the established breast cancer model. We have established by NMR experiments that the WW-domain of the prolyl isomerase Pin-1 which is required for breast cancer development in mice is a ligand for phosphorylated TF cytoplasmic domain. It is therefore possible that in breast cancer, the TF cytoplasmic domain contributes to tumor development. In conclusion, we established a breast cancer model that is suitable to study TF enhanced breast cancer growth, but this model did not show a suppression of breast cancer development by TF cytoplasmic domain signaling.

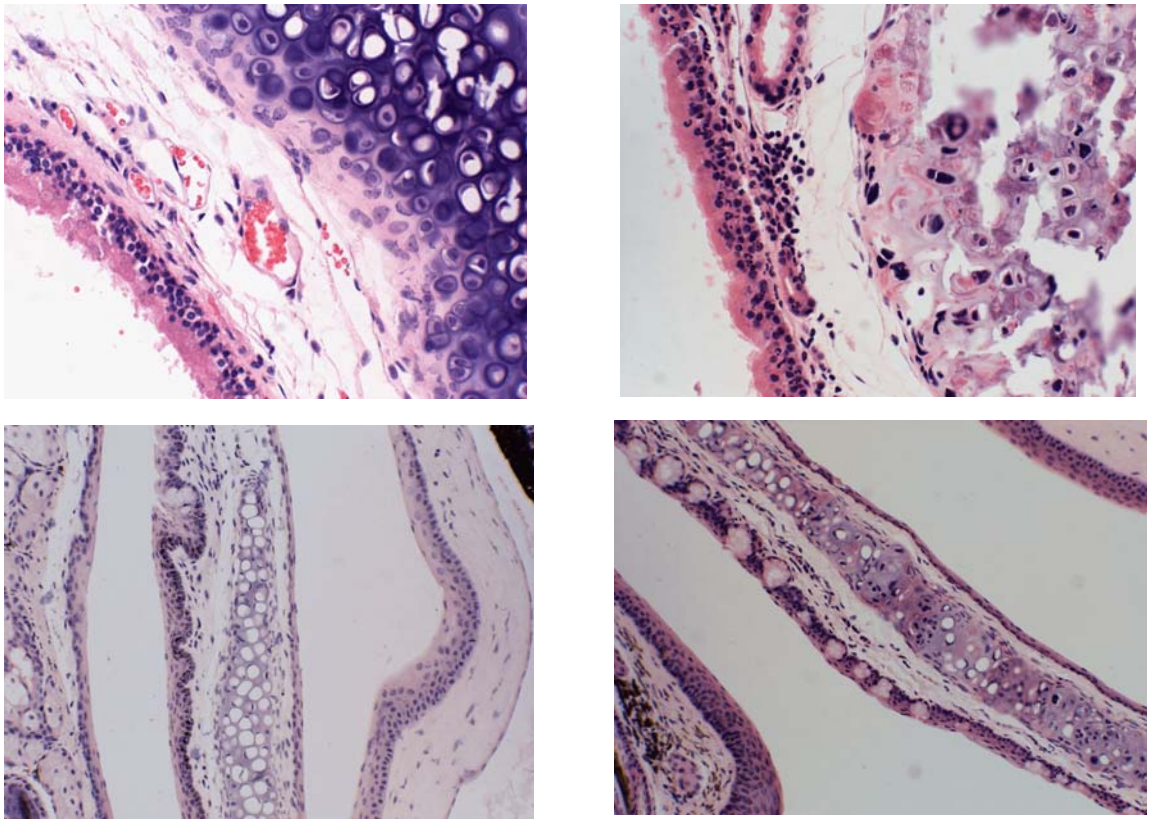

Fig. 2: Pathological cartilage in trachea (upper panels) and eyelids (lower panels) of wild-type (left) and C3-TAg (right) C57BL/6 mice.

Aim 2 is to generate tumor prone animals that lack the TF cytoplasmic domain. The first strategy was to cross hormone regulated C3-TAg mice with TF cytoplasmic domain deleted ($TF^{\Delta CD\Delta CD}$) mice. As proposed, we generated 56 mutant and 40 littermate-derived wild-type control mice that were transgene carriers and followed the cohort. Cancer free survival was up to 9 month which is longer than described in the literature for the same transgene on the FVB/N background. Most mice died from progressive weight loss between 7-9 months of age. Pathological examination revealed that these mice suffered from widespread cartilage dysplasia (achondroplasia, chondrodysplasia) in all major sites, including articular cartilage, growth plates of long bones and trachea. Fig. 2 shows examples of normal and C3-TAg trachea and eyelid cartilage. This pathology has recently been attributed to a rearrangement of the insertion site of the transgene during the backcrossing into the C57BL6 strain (Toxicol Pathol. (2004) 32:22-5).

We had originally proposed to use FVB/N-TgN(MMTVneu)202Mul mice as an alternative strategy. In considering the difficulties to control for strain effects in immunocompetent mice resulting from the cross of the FVB strain with $TF^{\Delta CD\Delta CD}$ in the C57BL/6 background, we decided to follow an alternative strategy. PyMT mice became available last year to us in the C57BL/6 genetic background. Breast cancer development in this model has been documented and the breast cancer pathology of polyoma middle T transgenic animals mirrors the stages of human breast cancer progression. We have generated the crosses between $TF^{\Delta CD\Delta CD}$ mice and PyMT mice in the C57BL6 background in the last year. We are approaching a cohort size of 20 animals that will be followed as originally proposed. We expect to have the analysis completed by the end of this calender year.

Key Research Accomplishments

- Demonstrated enhanced tumor growth upon TF expression in breast cancer cells using a tetracyclin regulated expression cassette
- Generated and followed $TF^{\Delta CD/\Delta CD}$/C3-TAg cohort. A strain specific cartilages pathology in the C57BL/6 C3-TAg transgenic line was detected and tumor progression could not be analyzed due to the limited lifespan of the animals
- Generated $TF^{\Delta CD/\Delta CD}$/PyMT C57BL/6 mice and established a cohort with the goal to characterize breast cancer development in 20 mice in comparison to 20 wild-type mice

Reportable Outcomes

Versteeg, H. H. , Mueller, B. M.., and Ruf, W. The role of tissue factor in breast cancer. Era of Hope meeting for the Department of Defense (DOD) Breast Cancer Research Program (BCRP), June 8-11, 2005, Philadelphia, Pennsylvania.

Conclusions

We have identified a strain specific reduction in breast cancer development in C3-TAg mice. We have acquired strain matched PyMT mice that in preliminary analysis develop tumors within the expected time frames of experiments published with this strain. Completion of these experiments will provide conclusive evidence for a role of the TF cytoplasmic domain in breast cancer progression.